



Genetic susceptibility to iatrogenic malignancy

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Iatrogenic malignancies represent a devastating and often fatal long-term effect of therapy administered for a prior condition, usually a primary cancer. Earlier diagnosis and the development of more effective cancer treatments over the last 30 years have considerably improved the long-term survival of patients. However, the burgeoning number of cancer survivors has led to a parallel increase in the number of cases of iatrogenic malignancy. Consequently, understanding host susceptibility factors, such that high-risk patients can be identified, has become a priority. However, this task is made difficult by the heterogeneity of iatrogenic malignancies. Nevertheless, the identification of polymorphic loci and pathways predicted to modify dose (e.g., glutathione S-transferases, nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase, cytochrome P450, and thiopurine S-methyltransferase) or determine cellular outcome (e.g., nucleotide excision DNA repair, base excision DNA repair, DNA mismatch repair, and cell death signaling) after therapy has provided insight into how host genetics may impact on the risk of developing iatrogenic malignancy.

Cancers arising secondary to diagnostic or therapeutic exposures can be defined as iatrogenic malignancies. The majority of such malignancies arise following chemotherapy and/or radiotherapy for a prior cancer. Iatrogenic malignancies may be particularly aggressive compared to *de novo* cancers arising in the same tissue. For example, therapy-related myeloid leukemia is associated with unfavorable cytogenetics and the overall survival is shorter than for sporadic disease of the same karyotypes [1,2]. Understanding host susceptibility factors may allow for the identification of individuals at high risk, enabling preventive and/or monitoring measures to be implemented. The study of susceptibility factors for second malignancy has traditionally focused on age, sex, type of primary cancer, and type of therapy [3]. However, the field has recently expanded to include attempts at understanding the role of host genetics in defining susceptibility to iatrogenic malignancy. In order to do this it is important to identify susceptibility loci and alleles, and establish how these interact with exposure to affect cellular response to therapeutic exposures and the subsequent risk of disease. It is also important to recognize the somewhat unique approaches that must be applied to the study of iatrogenic disease. This review will focus on addressing these issues.

Genetic epidemiology of iatrogenic malignancy

Efforts to identify host genetic susceptibility factors for iatrogenic malignancies have focused on

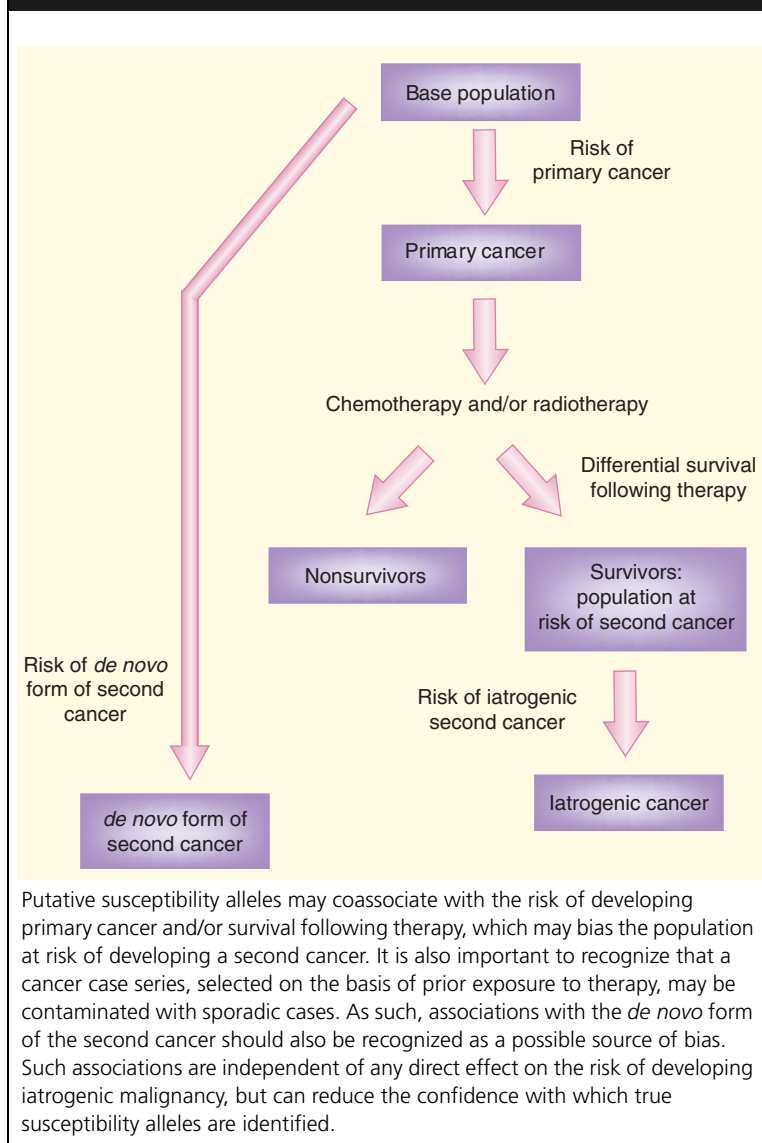
establishing case series and using these in the context of association studies. However, this approach, while successfully applied to the study of sporadic cancers, can be complicated by problems unique to the study of iatrogenic malignancy. Specifically, it is important to recognize that the population at risk of developing iatrogenic malignancy may be biased (not representative of the general population) by associations with risk factors for the primary condition which indicated the therapy (Figure 1). Differential survival rates following treatment for a primary cancer may also bias the population at risk of iatrogenic malignancy (Figure 1). Indeed, it is likely that the same loci, by nature of their interaction with specific chemotherapies or radiotherapies, will affect how both target tumor cells and nontumor cells (including the susceptible cell for transformation) respond to high-dose therapy. This raises an important question; does the overrepresentation of a putative susceptibility allele in a case-series of individuals with iatrogenic malignancy represent a true association with risk of that malignancy, or is it merely a reflection of differential survival and bias in the population at risk of iatrogenic malignancy?

It is also important to recognize a potential association between a putative susceptibility allele and the risk of developing the sporadic or *de novo* form of the iatrogenic cancer (Figure 1). Indeed, it is likely that any case series of individuals selected on the basis of prior exposure to carcinogenic therapies will include sporadic

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Figure 1. Identifying susceptibility alleles for iatrogenic malignancy can be made difficult by population bias.



cases, a problem that may be more acute when studying tumors at sites where therapy gives rise to only a modestly increased relative risk of cancer, such as stomach cancer after Hodgkin's disease (observed/expected = 1.9) [4]. Studies looking at genetic susceptibility to iatrogenic malignancy have focused predominantly on acute myeloid leukemia for chemotherapy exposures, where the relative risk is high, in addition to solid cancers for radiotherapy, where relative risk may be low but absolute excess risk is high. At sites where the relative risk of disease is very high, a case series is likely to be comprised predominantly of individuals whose cancer is related to therapy, thus increasing the power to

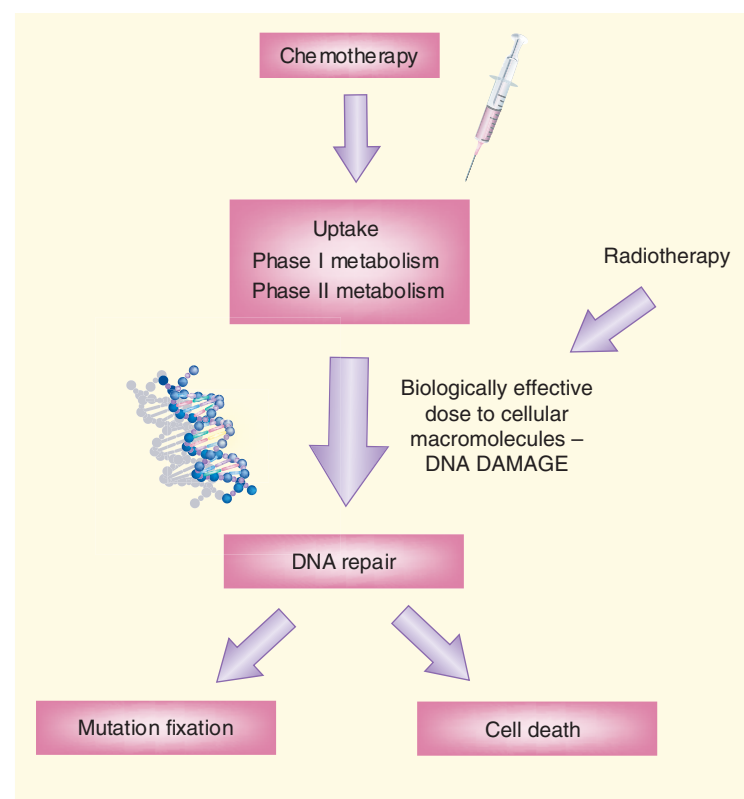
identify host genetic susceptibility factors. Unfortunately, at such cancer sites, in the bone marrow, for example, there tends to be a low absolute excess risk of therapy-induced disease. As such, establishing a case series of sufficient size and power in order to perform genetic studies can prove difficult.

Identifying susceptibility loci and alleles for iatrogenic cancers

Susceptibility alleles, defined by gene polymorphisms, can be characterized based on their frequency and functionality. Constitutive gene polymorphisms are often stratified based on their prevalence in a target population into high (1–50%) and low frequency (< 1%) alleles. The latter are often referred to as constitutional mutations, rather than polymorphisms. In many ways this stratification is arbitrary, but it may ultimately affect the way that information on host genetic susceptibility to iatrogenic malignancy is applied in the clinical setting.

A number of approaches may be adopted in order to improve the confidence with which host genetic susceptibility factors for iatrogenic malignancy can be identified. For example, biological pathways can be selected for investigation based on known or predicted gene–exposure interactions. Such an approach allows a heterogeneous population of cases to be stratified by exposure to known or putative substrates; this method has been successfully applied to the study of genetic polymorphisms in Phase II metabolism, and DNA repair pathways and the risk of iatrogenic malignancy [5,6]. However, stratification by exposure may not always be possible or appropriate, particularly when a biological role has not been narrowly defined or when gene–exposure interactions are very speculative. The molecular features of iatrogenic malignancies can be used as clues to the mechanisms by which they were induced, and provide an alternative basis for stratification. For example, the generation of chromosome translocations requires DNA double strand break formation, implicating aberrant DNA strand break repair in their etiology. As such, genetic susceptibility conferred by variant DNA strand break repair may theoretically be stratified by the presence of a chromosome translocation. Indeed, this stratification approach has been applied to the study of genetic variation in the metabolism of chemotherapeutic topoisomerase poisons and the risk of myeloid leukemia with chromosome translocations involving the mixed lineage leukemia (*MLL*) gene on chromosome 11q23, which

Figure 2. Susceptibility loci for iatrogenic malignancy can be identified by their putative interaction with chemotherapy or radiotherapy.



Boxes indicate functions that may be modified by genetic polymorphisms.

are postulated to arise via exposure to DNA topoisomerase inhibitors [7] (discussed later).

The search for iatrogenic malignancy susceptibility loci has quite naturally been driven by known or putative interactions between candidate loci and carcinogenic therapies. Indeed, using this approach putative susceptibility loci can be broadly classified into two groups (Figure 2):

- Those that modify the biologically effective dose delivered to the target cell and the extent of resultant damage to cellular macromolecules (dose modifiers).
- Those that modify the cellular response to that damage (response modifiers).

Loci in the former group may encode proteins involved in chemotherapy uptake, activation and detoxification. Conversely, loci in the latter group may encode proteins involved in DNA repair, mutation fixation and cell death/apoptosis (Figure 2). This stratification is not arbitrary, but differentiates between dose-modifying loci,

where predicting the impact on cancer risk is relatively simple, and response-modifying loci, where predicting the outcome on cancer risk is somewhat harder.

Dose-modifying loci/pathways will be discussed first, including glutathione *S*-transferases (GSTs), nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase (NQO1), cytochrome P450s (CYPs), and thiopurine methyltransferase (TPMT). Response-modifying loci/pathways will be discussed second, and these include DNA repair and cell death signaling. Finally, we will discuss the relationship between cancer susceptibility syndromes and iatrogenic malignancy. While we have attempted to cite all published articles reporting data on genetic susceptibility to iatrogenic or therapy-related malignancy, we have concentrated our discussion on those which can be used to introduce putative biological mechanisms of carcinogenesis, or raise issues relating to study design.

Glutathione *S*-transferases

GSTs are Phase II metabolizing enzymes that detoxify potentially mutagenic and toxic DNA-reactive metabolites by conjugation to glutathione. There are several cytosolic families of GSTs, including GST θ (GSTT), μ (GSTM) and π (GSTP) [8]. Numerous chemotherapy drugs are known or suspected substrates for GSTs, predominantly GST π , including etoposide, chlorambucil, melphalan, cyclophosphamide, busulfan, ifosfamide, cisplatin derivatives, and adriamycin [9–15], the majority of which are known or suspected human carcinogens.

Independent gene deletions exist at both the *GSTM1* and *GSTT1* loci, resulting in a lack of active protein in approximately 50 and 20% of Caucasians, respectively [16,17]. Despite its demonstrated physiological importance, the available evidence suggests that *GSTM1* gene deletion does not associate with risk of iatrogenic malignancy, either in the case of leukemia after chemotherapy or breast cancer after radiotherapy (Table 1) [5,18]. Therapy-related leukemia has been noted to be more common in patients with a *GSTT1* gene deletion [5,19,20]. However, this association appears to be driven by an association with leukemia *per se* [21], and is not specific to prior therapy. Thus, the significant association with acute myeloid leukemia (AML) following cancer chemotherapy reported by Sasai and colleagues may be a reflection of the healthy comparison group used in their study [19] (Table 1). This conclusion is supported by the findings of Woo and co-workers

Table 1. Glutathione S-transferase polymorphisms and the risk of iatrogenic malignancy.

Locus	Primary cancer/iatrogenic cancer	Comparison group (N)	Stratification (N)	Odds ratio (95% confidence interval)	Ref.
GSTM1, gene deletion	Various/AML	Healthy noncancer controls (n = 43)	None (n = 18)	Carriers 1.0 (-) Null 1.03 (0.31–3.37)	[19]
	Various/AML or MDS	Healthy noncancer controls (n = 150)	None (n = 58)	Carriers 1.0 (-) Null 0.77 (0–1.66)	[36]
	Pediatric acute lymphoblastic leukemia/AML or MDS	2.5 year survivors of paediatric acute lymphoblastic leukemia (n = 245)	None (n = 57)	Carriers 1.0 (-) Null 0.93 (0.56–4.54)*	[22]
	Various/AML	<i>De novo</i> leukemia (n = 417)	None (n = 89)	Carriers 1.0 (-) Null 0.99 (0.62–1.60)	[5]
			Radiation (n = 38)	Carriers 1.0 (-) Null 1.25 (0.61–2.60)	
			Any chemotherapy (n = 51)	Carriers 1.0 (-) Null 0.85 (0.48–1.98)	
	Various/AML or MDS	Healthy noncancer controls (n = 239)	None (n = 44)	Carriers 1.0 (-) Null 1.52 (0.79–2.94)*	[20]
	Hodgkin's disease/various	5-year survivors of Hodgkin's disease (n = 646)	None (n = 127)	Carriers 1.0 (-) Null 1.4 (0.99–2.01)	[18]
			Any cancer in radiation field (n = 108)	Carriers 1.0 (-) Null 1.3 (0.92–1.98)	
	Hodgkin's disease/breast cancer	5-year survivors of Hodgkin's disease (n = 646)	Breast cancer in radiation field (n = 54)	Carriers 1.0 (-) Null 1.2 (0.67–2.23)	[18]
GSTT1, gene deletion	Various/AML	Healthy noncancer controls (n = 177)	None (n = 42)	Carriers 1.0 (-) Null 0.98 (0.48–1.97)	[64]
	Various/AML	Healthy noncancer controls (n = 43)	None (n = 18)	Carriers 1.0 (-) Null 4.62 (1.48–14.4)	[19]
	Pediatric acute lymphoblastic leukemia/AML or MDS	2.5-year survivors of pediatric acute lymphoblastic leukemia (n = 245)	None (n = 57)	Carriers 1.0 (-) Null 1.54 (0.80–2.96)*	[22]
	Various/AML	<i>De novo</i> leukemia (n = 417)	None (n = 89)	Carriers 1.0 (-) Null 1.19 (0.67–2.13)	[5]
			Radiotherapy (n = 38)	Carriers 1.0 (-) Null 0.66 (0.26–1.84)	
			Any chemotherapy (n = 51)	Carriers 1.0 (-) Null 1.61 (0.83–3.14)	
	Various/AML or MDS	Healthy noncancer controls (n = 239)	None (n = 44)	Carriers 1.0 (-) Null 1.98 (0.94–4.19)*	[20]
	Hodgkin's disease/various	5-year survivors of Hodgkin's disease (n = 646)	None (n = 127)	Carriers 1.0 (-) Null 0.9 (0.56–1.38)	[18]
			Any cancer in radiation field (n = 108)	Carriers 1.0 (-) Null 0.9 (0.53–1.41)	
	Hodgkin's disease/breast cancer	5-year survivors of Hodgkin's disease (n = 646)	Breast cancer in radiation field (n = 54)	Carriers 1.0 (-) Null 1.1 (0.53–2.17)	

*Odds ratios and 95% confidence intervals were not presented in the original report, and were calculated using the χ^2 test without adjustment.

AML: Acute myeloid leukemia; GSTM: Glutathione S-transferase μ ; GSTP: Glutathione S-transferase π ; GSTT: Glutathione S-transferase θ ; MDS: Myelodysplastic syndrome.

Table 1. (continued) Glutathione S-transferase polymorphisms and the risk of iatrogenic malignancy.

GSTP1, Ile–Val, codon 105	Various/AML	<i>De novo</i> leukemia (n = 414)	None (n = 89)	Ile/Ile 1.0 (-) Ile/Val 1.87 (1.11–3.17) Val/Val 1.67 (0.84–3.30)	[5]
			Radiotherapy (n = 38)	Ile/Ile 1.0 (-) Ile/Val 0.94 (0.42–2.12) Val/Val 1.16 (0.43–3.13)	
			Any chemotherapy (n = 51)	Ile/Ile 1.0 (-) Ile/Val 2.87 (1.45–5.67) Val/Val 2.17 (0.89–5.29)	
			GSTP1 substrates (n = 21)	Ile/Ile 1.0 (-) Ile/Val 4.43 (1.39–14.12) Val/Val 4.16 (1.07–16.07)	

*Odds ratios and 95% confidence intervals were not presented in the original report, and were calculated using the χ^2 test without adjustment.

AML: Acute myeloid leukemia; GSTM: Glutathione S-transferase m; GSTP: Glutathione S-transferase p; GSTT: Glutathione S-transferase q; MDS: Myelodysplastic syndrome.

who, using a comparison group matched on primary cancer and chemotherapy, reported no association between nullizygosity for *GSTT1* and *GSTM1* (absence of both genes) and the risk of acute myeloid leukemia or myelodysplasia in children treated with chemotherapy for acute lymphoblastic leukemia [22] (Table 1).

GSTP1, encoded by a single locus (*GSTP1*) on chromosome 11, is also subject to polymorphic variation [23]. In contrast to *GSTM1* and *GSTT1*, there is compelling evidence from one study supporting a direct association between the *GSTP1* isoleucine to valine substitution at codon 105 and the risk of developing an iatrogenic malignancy [5]. A weak association between leukemia risk and *GSTP1* codon 105 status is strengthened when the iatrogenic case series is restricted to individuals who had chemotherapy (Table 1), and is further strengthened when only those cases that were exposed to known GSTP1 substrates are included in the analysis (Table 1). The use of a *de novo* leukemia case series as a comparison group controls for any putative association with leukemia *per se*. However, an association between the codon 105 valine-encoding variant and improved survival after adriamycin and/or cyclophosphamide-based chemotherapy, for either breast cancer or Hodgkin's lymphoma, suggests a possible bias in the population at risk of developing subsequent malignancy [24,25].

The codon 105 variant may also confer susceptibility to solid cancers after chemotherapy for pediatric lymphoblastic leukemia. In a series of 16 cases with second cancer, Jazbec and colleagues reported six individuals (33%) who were homozygous for the rare codon 105 variant, whilst no homozygotes were reported in a series of 32 matched control patients who were treated

for the same primary malignancy but had not developed a second cancer [26].

The *GSTP1* codon 105 residue forms part of the GSTP1 active site which binds hydrophobic electrophiles [27], and the isoleucine–valine substitution affects substrate-specific catalytic activity and thermal stability of the encoded protein [9,28–30]. Thus, a demonstration of functionality provides further evidence for a role in defining susceptibility to chemotherapy-induced malignancy.

Metabolism of topoisomerase inhibitors

DNA topoisomerases function to maintain DNA topology by regulating supercoiling, catenation and knotting. This is achieved via a process that involves the transient cleavage of DNA. This property has led to the development of DNA topoisomerase inhibitors as anticancer chemotherapeutic agents. Unfortunately, many such agents, including the epipodophyllotoxins and anthracyclines, are also human carcinogens. Acute leukemia, characterized by translocations involving the *MLL* gene on chromosome 11q23, is common following therapy involving topoisomerase inhibitors [7]. Indeed, it is the inhibition of DNA topoisomerases by anthracyclines (adriamycin) and epipodophyllotoxins (etoposide and teniposide) that leads directly to *MLL* gene translocations [31–32].

DNA topoisomerase poisons, like other quinone-containing compounds, are subject to cellular metabolism. As such, pathways that modulate the metabolism and detoxification of quinone-containing chemotherapeutics have been investigated as putative modifiers of susceptibility to iatrogenic malignancy, including NQO1 (or DT-diaphorase) and the CYPs.

NQO1 catalyses the two-electron reduction of quinone-containing chemotherapeutics to form hydroquinone [33]. This reaction is in competition with a one-electron reduction catalyzed by CYPs, producing the semiquinone. It is important to note that the semiquinone can also undergo redox cycling to generate reactive oxygen species [34], which are thought to contribute to the carcinogenicity of quinone-containing chemotherapeutics independent of their activity against DNA topoisomerases. Polymorphic variation in *NQO1* has been investigated as a potential modifier of leukemia risk following treatment with topoisomerase inhibitors. An association between the inactivating C>T polymorphism at nucleotide position 609 (codon187 Pro>Ser) of *NQO1* and risk of leukemia after topoisomerase inhibitors has been reported by two groups [35–36] (Table 2). However, the reported associations were not specific to prior chemotherapy with topoisomerase poisons, suggesting a possible association with leukemia *per se*. Furthermore, subsequent studies report no association between the codon 187 *NQO1* polymorphism and therapy-related leukemia, even when restricted to *MLL* translocation-positive cases [37–39] (Table 2). As such, while there is clear evidence supporting chemotherapeutic topoisomerase inhibition as a cause of *MLL* gene aberrations, the role of *NQO1* as a modifier of this effect remains less clear.

MLL itself has also been investigated for genetic variation that may predispose to translocation. Using a case series of 22 individuals who developed 11q23 translocation-positive acute leukemia after treatment with topoisomerase inhibitors, Echlin-Bell and colleagues identified numerous polymorphic microsatellite repeats elements within *MLL*, but none were significantly associated with subsequent risk of translocation-positive leukemia [40].

CYP polymorphisms have also been investigated as potential modifiers of leukemia risk following chemotherapy. Indeed, a role in the metabolism of etoposide, teniposide and other chemotherapeutic agents makes the CYPs likely modifiers of iatrogenic cancer risk. Using a case series comprised predominantly of individuals with prior exposure to topoisomerase inhibitors and with 11q23 (*MLL*) translocation-positive myeloid or lymphoblastic leukemia, Felix and co-workers reported an apparently protective association between a polymorphism in the promoter of the *CYP3A4* gene and the risk of therapy-related disease (Table 2) [41]. An inverse association

between this polymorphism and the T-cell receptor gene V γ /J β rearrangement, a marker of genotoxicity, in the circulating lymphocytes of children undergoing chemotherapy for lymphoblastic leukemia further supports a role for *CYP3A4* as a modifier of iatrogenic cancer risk [42]. However, a pediatric case-control study failed to reproduce the association between *CYP3A4* status and myeloid leukemia arising after antitopoisomerase-based chemotherapy for acute lymphoblastic leukemia [38]. Indeed, the frequency of the variant allele was actually higher in *MLL* translocation-positive therapy-related leukemia cases compared with translocation-negative cases.

Polymorphisms in other CYP genes have also been investigated as potential modifiers of iatrogenic cancer risk, including *CYP2D6*, *CYP2C19* and *CYP3A5*, although there is no evidence supporting any association [38,43].

Thiopurine methyltransferase

Thiopurine prodrugs, such as 6-thioguanine, 6-mercaptopurine and azathioprine, are used extensively to treat both cancerous and noncancerous conditions. Their biological activity is dependent on activation to form thioguanine nucleotides, which can subsequently be incorporated into nucleic acids. Thioguanine nucleotides are subject to S-methylation and detoxification by TPMT yielding methylated thioguanine nucleotides, which are biologically inactive. In humans, TPMT activity is highly variable, with approximately 90% of individuals having high activity, 10% with intermediate activity and 0.3% having low or null activity. This phenotypic heterogeneity is the result of a high degree of polymorphism in the *TPMT* gene (reviewed in [44]). To date, eight *TPMT* alleles have been identified, with three of these (*TPMT2*, *TPMT3A* and *TPMT3C*) accounting for approximately 90% of all intermediate-, low- and null-activity cases. Individuals homozygous or compound heterozygous for either *TPMT2*, *TPMT3A* or *TPMT3C* are null for TPMT activity, whereas heterozygotes with one wild-type allele have intermediate TPMT activity [45]. TPMT activity is a critical determinant of patient response to thiopurine-based therapy. Indeed, treatment of low- or null-activity patients with standard dose therapy can lead to acute bone marrow toxicity, or even death, due to neutropenia [46,47]. Furthermore, mild toxicity has also been reported in some heterozygotes, demonstrating allelic haploinsufficiency, at least in the context of treatment with thiopurine chemotherapeutics [48,49]. Recent data clearly demonstrates

Table 2. Genes involved in the metabolism of topoisomerase inhibitors and risk of iatrogenic malignancy.

Locus	Primary cancer/iatrogenic cancer	Comparison group (N)	Stratification (N)	Odds ratio (95% confidence interval)	Ref.
NQO1, Pro-Ser, codon 187	Various/AML	<i>De novo</i> acute leukemia and MDS (n = 48)	None (n = 56)	Pro/Pro 1.0 (-) Pro/Ser 0.93 (0.42–2.07)* Ser/Ser 2.67 (0.49–14.49)*	[35]
			Topoisomerase inhibitors (n = 27)	Pro/Pro 1.0 (-) Pro/Ser 0.92 (0.34–2.48)* Ser/Ser 2.77 (0.41–18.75)*	
	Various/AML or MDS	Noncancer controls (n = 150)	None (n = 58)	Pro/Pro 1.0 (-) Pro/Ser 0.98 (0.28–1.68) Ser/Ser 2.62 (2.16–3.08)	[36]
	Various/MLL translocation +ve acute leukemia (lymphoid and myeloid)	MLL translocation -ve <i>de novo</i> B-cell acute lymphoblastic leukemia (n = 56)	11q23 (MLL) translocation +ve (n = 18)	Pro/Pro 1.0 (-) (Pro/Ser + Ser/Ser) 0.59 (0.19–1.85)	[37]
	Paediatric lymphoblastic leukemia/myeloid leukemia or MDS	4-year survivors of paediatric lymphoblastic leukemia (n = 160)	Caucasians (n = 41)	Pro/pro 1.0 (-) (Pro/Ser + Ser/Ser) 1.45 (0.73–2.92)	[38]
	Various/AML	Noncancer controls (n = 175)	None (n = 33)	Pro/Pro 1.0 (-) Pro/Ser 1.62 (0.74–3.54) Ser/Ser – (no cases in t-AML series)	[39]
CYP3A4, A>G -288 promoter	Various/acute leukemia (myeloid and lymphoblastic)	<i>De novo</i> acute leukemia (myeloid and lymphoblastic) (n = 99)	None (n = 30)	wt/wt 1.0 (-) (wt/v + v/v) 0.09 (0.01–0.87)	[41]
			11q23 (MLL) translocation +ve (n = 22)	wt/wt 1.0 (-) (wt/v + v/v) 0.09 (0.01–1.58)	
	Pediatric lymphoblastic leukemia/myeloid leukemia or MDS	4-year survivors of pediatric acute lymphoblastic leukemia (n = 167)	Caucasians (n = 41)	wt/wt 1.0 (-) (wt/v + v/v) 1.53 (0.46–5.09)	[38]

*Odds ratios and 95% confidence intervals were not presented in the original report, and were calculated using χ^2 test without adjustment.

AML: Acute myeloid leukemia; CYP: Cytochrome P450; MDS: Myelodysplastic syndrome; MLL: Mixed lineage leukemia; NQO: Nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase; t-AML: Therapy-related acute myeloid leukemia.

the importance of TPMT genotype and phenotype on clinical outcome after thiopurine therapy for both arthritis and lymphoblastic leukemia [50].

There is accumulating evidence to suggest that TPMT status may also play a critical role in modulating iatrogenic cancer risk after thiopurine therapy. An unusually high frequency of brain tumors in children treated for lymphoblastic leukemia with cranial radiotherapy and high-dose 6-mercaptopurine prompted an investigation into the potential causes [51]. Three of the six children who developed a brain tumor were subsequently confirmed as polymorphic at the TPMT locus (two heterozygotes and one homozygote for low-activity alleles), equating to an 8-year cumulative inci-

dence of brain tumors in carriers of TPMT polymorphisms of 43%, compared with just 8% for children homozygous for the common wild-type allele [51]. Moreover, a fourth child diagnosed with a brain tumor had phenotypically intermediate TPMT activity, although was not genetically polymorphic for TPMT, suggesting the possible existence of other as yet unidentified genetic determinants of TPMT activity. Another study reported an association between the risk of acute myeloid leukemia following 6-mercaptopurine-based therapy, also given for pediatric lymphoblastic leukemia, and TPMT genotype [52]. Four out of 384 patients treated for lymphoblastic leukemia subsequently developed myeloid leukemia, and

two of these four patients were genotyped as heterozygous for the *TPMT3A* allele [52], a frequency of heterozygosity substantially higher than expected in the general population (10%). There was also a clear association between TPMT phenotype, determined in all study subjects using red blood cell TPMT activity, and the risk of subsequent myeloid leukemia.

Taken together, these data provide compelling evidence in support of TPMT status, as determined either by gene polymorphism or phenotype, as a modifier of thiopurine therapy-related cancer. It will be of interest to determine if TPMT status is also a modifier of cancer risk after organ transplant, where thiopurine immunosuppressants, such as azathioprine, are used to protect against transplant rejection but are implicated as carcinogens [53,54].

DNA repair and cell death signaling

DNA repair is essential for the maintenance of genomic stability, and functions to suppress tumor formation. Critically, numerous therapeutic agents cause cancer via a mechanism that involves the induction of DNA damage, including alkylating agents, topoisomerase inhibitors and radiotherapy. As such, heterogeneity in the efficiency and fidelity of DNA repair, as conferred by constitutive genetic polymorphism, is a potential modifier of susceptibility to iatrogenic malignancy.

There is considerable evidence to suggest that the polymorphic xeroderma pigmentosum group D gene (*XPD*), a component of the nucleotide excision repair pathway, affects cellular response to chemotherapy and the risk of subsequent cancer (Table 3). A modest but significant association was reported between homozygosity for the glutamine-encoding allele at codon 751 and an increased risk of leukemia following chemotherapy, but not radiotherapy, for a prior condition (Table 3) [55]. These data are consistent with a role for nucleotide excision repair in mediating cellular response to DNA damage induced by some chemotherapy agents [56,57], but not in response to radiation-induced damage. The glutamine allele was also found to be significantly associated with the risk of developing a second primary cancer after either basal cell or squamous cell carcinoma of the skin [58]. Although radiotherapy and topical chemotherapy may be used in the treatment of non-melanoma skin cancer, the use of systemic chemotherapy is extremely rare. As such, the association between the *XPD* codon 751 polymorphism and second cancer after nonmelanoma skin cancer may suggest a risk for

malignancy *per se* independent of any treatment. Nevertheless, a putative role for the codon 751 *XPD* polymorphism in modifying cellular response to chemotherapy is substantiated by its confirmation as an independent prognostic marker for both colon cancer and leukemia in patients treated with chemotherapy-based protocols [55,59].

The diverse cellular functions of *XPD* have led to speculation regarding potential functionality of the codon 751 polymorphism. One model predicts a direct influence on DNA repair capacity, where the codon 751 polymorphism may have an effect on the repair of either promutagenic or protoxic DNA lesions, or possibly both. In this model, predicting the overall effect on cancer risk is made difficult because repair of promutagenic lesions will be protective, whereas repair of protoxic lesions could theoretically confer susceptibility, by preventing elimination of mutagenized cells via apoptosis. Indeed, this model illustrates how genetic variation may conceivably affect iatrogenic cancer risk without modulating the biologically-effective dose, by means of affecting the cellular response to exposure.

Under conditions of extreme genotoxic stress, an inability to initiate cell death when appropriate could lead to cellular transformation. Indeed, it is this mechanism that is thought to at least partly contribute to malignant transformation in mice deficient in DNA mismatch repair following treatment with carcinogens [60]. This hypothesis prompted Worrillow and colleagues to investigate polymorphic mutS homolog 2 (*MSH2*), a major component of DNA mismatch repair, as a risk factor for therapy-related leukemia [6]. Certain leukemogenic alkylating agents, including procarbazine and cyclophosphamide, are known to attack the O⁶-position of guanine, generating O⁶-alkylguanine, a DNA lesion that signals cell death via interaction with *MSH2*. Consistent with a role in modulating susceptibility to chemotherapy-induced leukemia, a polymorphism in the splice acceptor region of *MSH2* intron 12 was significantly overrepresented in therapy-related AML (t-AML) cases, but only in those cases with previous exposure to O⁶-guanine alkylating chemotherapy agents (Table 3) [6]. In this particular model, the probability of surviving cells undergoing malignant transformation may be further enhanced by the extreme mutability of O⁶-alkylguanine DNA lesions [61].

Other polymorphic DNA repair genes have also been investigated in single studies as potential modifiers of iatrogenic cancer risk following either therapeutic or diagnostic exposures,

Table 3. DNA repair gene polymorphisms and risk of iatrogenic malignancy.

Locus	Primary cancer/iatrogenic cancer	Comparison group (N)	Stratification (N)	Odds ratio (95% confidence interval)	Ref.
<i>XRCC1</i>, Arg–Pro, codon 399	Various/AML	Noncancer controls (n = 178)	None (n = 34)	Arg/Arg 1.0 (-) Arg/Pro 0.54 (0.24–1.23) Pro/Pro 0.28 (0.09–0.88)	[39]
	Hodgkin's disease/various (basal cell carcinoma, breast cancer)	5-year survivors of Hodgkin's disease (n = 644)	Cancer in radiation field (n = 107)	Arg/Arg 1.0 (-) Arg/Pro 1.1 (0.69–1.59) Pro/Pro 1.1 (0.63–2.02)	[18]
<i>XPD</i>, Lys–Gln, codon 751	Various/AML	Noncancer controls (n = 73)	None (n = 15)	Lys/Lys 1.0 (-) Lys/Gln 9.66 (0.78–119.57) Gln/Gln 1.13 (0.04–28.8)	[39]
	Various/AML	Noncancer controls (n = 729)	Chemotherapy (n = 51)	Lys/Lys 1.0 (-) Lys/Gln 1.22 (0.63–2.36) Gln/Gln 2.22 (1.04–4.74)	[55]
	Basal or squamous cell carcinoma of the skin/various	Survivors of basal or squamous cell carcinoma (n = 401)	None (n = 80)	Lys/Lys 1.0 (-) Lys/Gln 2.27 (1.32–3.91) Gln/Gln 1.98 (0.93–4.21)	[58]
<i>MSH2</i>, -6 T>C exon 13	Various/AML	Noncancer controls (n = 776)	Chemotherapy (n = 50)	TT 1.0 (-) TC 1.40 (0.63–3.08) CC 2.81 (0.61–13.03)	[6]
			O ⁶ -guanine alkylating agents (n = 16)	TT 1.0 (-) TC 3.81 (1.26–11.48) CC 5.55 (0.65–47.67)	
<i>RAD51</i>, -135 G>C 5'UTR	Various/AML	Noncancer controls (n = 186)	None (n = 51)	GG 1.0 (-) GC 2.84 (1.24–6.51) CC – (no cases in t-AML series)	[64]

AML: Acute myeloid leukemia; *MSH2*: MutS homolog 2; UTR: Untranslated region; *XPD*: Xeroderma pigmentosum; *XRCC*: Excision repair cross-complementing.

including *XRCC3*, *RAD51*, *APE*, *MLH1*, *MSH3*, and *XRCC1* [39,62–64]. Positive associations have been reported for the *RAD51* -135 5' untranslated region polymorphism and the *XRCC1* codon 399 polymorphism and risk of t-AML (Table 3) [39,64], although the latter is not associated with risk of solid cancer after radiotherapy for Hodgkin's disease [18] (Table 3). Nevertheless, both genes encode proteins that function in mediating cellular response to radiotherapy and chemotherapy, and polymorphic variation remains a likely modifier of iatrogenic malignancy risk. The confirmed functionality of polymorphisms in AP endonuclease (*APE*) [65,66], a component of base excision repair, also make these likely modifiers of iatrogenic malignancy risk worthy of investigation.

Familial cancer genes

Neurofibromatosis type 1 (NF) is an autosomal dominant disorder characterized by neurofibro-

mas, which are benign tumors of the nerve sheath, and is the result of constitutive monoallelic mutation or deletion of the *NF1* gene. NF patients are also predisposed to numerous malignant cancers, including AML [67]. Several different *NF1* mutations have been identified, but none predominate. Consistent with its role as a tumor suppressor gene, biallelic inactivation or deletion of *NF1* is often observed in cancers arising in NF patients, including leukemia [68–70]. However, there is some debate as to whether heterozygosity and putative associated haploinsufficiency may confer susceptibility to therapy-related malignancy. Maris and co-workers [71] reported the occurrence of a second malignant disorder in five out of seven NF children treated with chemotherapy for a primary cancer, a frequency substantially higher than that expected in untreated NF children (approximately 10%) [67]. Second malignant disorders included three cases of myelodysplasia, one case of myeloid leukemia

and one patient who developed both myeloid leukemia and medulloblastoma. Intriguingly, genetic analysis demonstrated retention of heterozygosity of *NF1* in the second malignant disorders of four cases (the fifth case was noninformative), suggesting a susceptibility to iatrogenic cancer induction conferred by heterozygosity and concomitant haploinsufficiency, rather than through loss of *NF1* tumor suppressor function. Consistent with a role for *NF1* allelic loss in conferring susceptibility to therapy-induced cancer, heterozygous (*Nf1* +/-) mice display acute sensitivity to the leukemogenic effects of both etoposide and cyclophosphamide at doses that fail to induce malignancy in wild-type mice [72]. However, in contrast to the data reported by Maris and colleagues in human NF [71], murine leukemias invariably showed *Nf1* loss of heterozygosity [72], suggesting that susceptibility is mediated by chemotherapy-induced allelic inactivation, selection of *NF1* null cells and loss of tumor suppression function, rather than heterozygosity and haploinsufficiency giving rise to a classical modifier effect. Thus, although monoallelic *NF1* mutation appears to confer susceptibility to therapy-induced leukemia, the specific mechanism of action in humans remains to be determined.

Li-Fraumeni syndrome, like NF, is an autosomal dominant disorder characterized by predisposition to both sporadic and iatrogenic cancers [73,74], although a high frequency of spontaneously developing multiple primary cancers [75] can make it difficult to assess the extent of susceptibility to iatrogenic malignancy. A role for *P53*, the affected gene in Li-Fraumeni syndrome, in mediating cellular response to chemotherapy and radiotherapy-induced DNA damage provides biological plausibility for a link with iatrogenic malignancy. Moreover, common nonpathogenic polymorphisms at codon 47 and codon 72 that affect cellular apoptotic potential [76,77] further supports *P53* as a candidate modifier of iatrogenic cancer risk.

In contrast to the dominant negative nature of neurofibromatosis and Li-Fraumeni inheritance, there are several human cancer susceptibility syndromes where inheritance is autosomally recessive. Many of these syndromes, such as ataxia telangiectasia (AT), for example, are associated with defects in genes that operate in DNA damage response or repair pathways, and confer susceptibility to sporadic cancer. AT is a very rare disorder caused by constitutional biallelic mutation of the *ATM* gene. Although yet to be formally tested, the acute sensitivity of cells from

AT patients to radiation-induced clastogenesis [78,79] and reports of post-therapy cancer in AT patients [80,81] suggest an associated susceptibility to iatrogenic malignancy. As such, we can hypothesize that heterozygosity (or carrier status) may also confer susceptibility to iatrogenic malignancy; approximately 1% of the general population are heterozygous [82]. Cells from AT heterozygotes are moderately sensitive to the clastogenic effects of ionizing radiation compared with wild-type cells [78,79,83]. These data are consistent with mouse studies demonstrating increased sensitivity to radiation oncogenesis and death in AT heterozygous (*Atm* +/-) cells and animals [84,85], although the phenotype appears to be relatively modest. Despite the convincing laboratory data there is little evidence supporting an association between radiation carcinogenesis and *ATM* heterozygosity in humans, where studies have concentrated on radiogenic breast cancer after treatment for Hodgkin's disease [86–89]. Nevertheless, given the putative association with sporadic breast cancer [90–93] and reports of acute adverse response to radiotherapy [94], it remains possible that heterozygosity for pathogenic *ATM* mutations may also confer susceptibility to radiogenic breast cancer, although the penetrance appears to be low. We must also consider the possibility that nonpathogenic *ATM* polymorphisms (those that do not cause AT when inherited in the homozygous state or as a component in compound heterozygosity), with some previously associated with sporadic cancer risk and radiosensitivity [95], may also modify the risk of developing iatrogenic cancer.

Like AT, Fanconi anemia (FA) and Nijmegen breakage syndrome (NBS) are also autosomal recessive disorders characterized by cancer susceptibility. Acute sensitivity to the clastogenic and mutagenic effects of ionizing radiation suggests that FA and NBS patients may also be susceptible to iatrogenic malignancy [96,97]. However, reports of such cancers are rare [98], which is likely to be a reflection of the acute toxicity associated with cancer therapy and the concomitantly poor prognosis of FA and NBS patients. Nevertheless, cellular sensitivity to the clastogenic effects of chemotherapy/radiotherapy and/or cancer predisposition in carriers of pathogenic mutations associated with FA [99–101] and NBS [78,102] implicates heterozygosity for the causative mutations as potential modifiers of iatrogenic cancer risk. Again, as for *ATM*, a role in mediating cellular response to DNA damage for the *FA* genes and *NBS1*, the gene affected in

Highlights

- Genetic susceptibility studies have focused on chemotherapy-related leukemia and radiogenic solid cancers.
- The population at risk of iatrogenic malignancy may not be representative of the general population, necessitating the careful selection of comparison or control groups for genetic epidemiology studies.
- Evidence supports a role for polymorphic glutathione S-transferase P1 as a modifier of iatrogenic cancer risk, either directly or by affecting survival after chemotherapy.
- Initial evidence suggests that thiopurine methyltransferase polymorphisms modify cancer risk after thiopurine-based chemotherapy.
- Both promutagenic and antiapoptotic phenotypes may contribute towards an increased risk of therapy-induced cancer.
- Autosomally dominant human disorders, such as neurofibromatosis and Li-Fraumeni syndrome, predispose to both sporadic and iatrogenic cancers.
- Carrier status (heterozygosity) for the autosomally recessive human disorders ataxia telangiectasia, Fanconi anemia and Nijmegen breakage syndrome may predispose to iatrogenic malignancy, although the penetrance would appear to be relatively low.

NBS, provides biological plausibility for a role in modulating the risk of iatrogenic malignancy. Susceptibility to radiogenic cancer in mice heterozygous for *Nbn*, the mouse homolog of *NBS1*, further supports a potential role for *NBS1* heterozygosity as a modifier of iatrogenic malignancy risk in humans [103].

Future directions

It is clear that host genetics play a critical role in defining the risk of iatrogenic malignancy.

However, it is likely that the genetic contribution to defining iatrogenic cancer risk is polygenic. As such, accurately estimating the contribution of single gene variants in this context will require large well-controlled studies, with sufficient power such that specific gene–exposure interactions, and the influence of age and gender, can be investigated. Accurate estimates of associated risk would need to be made before risk management could be routinely applied clinically, either to offer post-therapy surveillance or alternative therapies, where possible, to high-risk patients. Risk management could prove particularly important in children or young adults with primary cancers, where cure rates can be high and the risk of subsequent iatrogenic cancer is a serious consideration.

Iatrogenic malignancies provide a unique opportunity in which to study the causal links between exposure and malignant transformation, and how constitutional genetics may modify this relationship. Therefore, at the very least, the study of such malignancies will further contribute to the understanding of molecular carcinogenesis that is also likely to apply to sporadic cancers.

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